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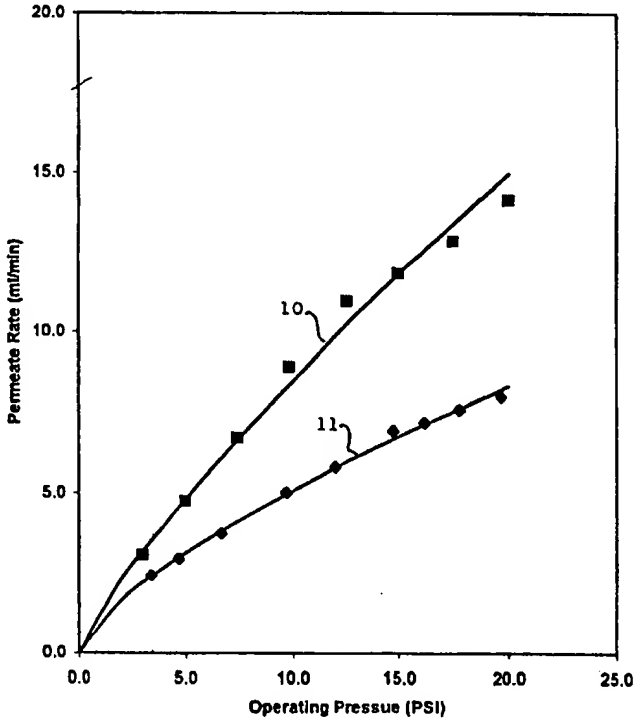
(54) Title: PLASMA-ANNEALED MEMBRANE FOR PROTEIN SEPARATION

(57) Abstract

Improved porous polymeric membranes, annealed by a process of plasma-annealing, are used in separations and concentrative applications involving solutions or suspensions containing proteinaceous substances. Thus, porous membranes, which were treated by exposure to a glow discharge through a gas mixture containing an alkane such as methane in combination with oxygen, air, or a hydrophilic unsaturated organic monomer, exhibit greatly improved flux toward protein-containing fluids, and reduced protein fouling and loss due to adsorption on membrane surfaces. The plasma-annealed membranes are useful in fractionations and separations involving protein-containing fluids.



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Plasma-Annealed Membrane for Protein Separation

Technical Field

This invention relates to porous membranes. More specifically, this invention relates to use of plasma-annealed porous membranes in separation and concentration of proteinaceous solutions and suspensions.

Background Art

Porous materials have been increasingly applied to processing of solutions containing biological matter. These processes may involve filtration, clarification, fractionation, pervaporation, reverse osmosis, dialysis, affinity separation, blood oxygenation, or similarly related procedures. A common occurrence in such processes is loss of process efficiency due to fouling of material surfaces by biological matter, and most particularly by proteinaceous matter. Such fouling robs these processes of their efficiency and cost effectiveness, entailing process downtime for cleaning and early replacement of irretrievably fouled portions of porous material components.

Proteinaceous biomolecules are highly complex, containing both hydrophilic and hydrophobic regions. These biomolecules are highly conformable and adaptable toward adsorption to surfaces having hydrophobic moieties thereat. They are inherently surface-active and readily bind to material surfaces at the surface-liquid interface. The problem of uncontrolled adsorption of proteinaceous matter extends through a myriad of processes and applications involving contact or processing of proteinaceous solutions and suspensions.

Ultrafiltration of whey through microporous polysulfone membranes provides a particularly prominent example of this problem. Ultrafiltration membranes typically show extremely high throughputs of water when tested on pure water as a process feedstream. Contact with a whey as a process feedstream almost always results in a prompt and drastic decline in flux through the same membrane. Adsorption of proteinaceous matter, particularly beta-lactoglobulin and alpha-lactalbumin, has been identified as a primary contributing cause for such drastic flux declines.

Other examples of deleterious effects of protein adsorption on porous surfaces include: clotting of blood on dialysis and oxygenator membranes and hemolysis of red blood cells on surfaces; loss of costly bioengineered proteins (growth hormones,

clotting factors, specialty enzymes, etc.) due to irreversible adsorption onto processing membranes; inability to size-fractionate proteins by filtration due to concomitant adsorption and fouling of filter surfaces by proteinaceous molecules; and high background noise in some types of DNA/polynucleic acid blotting analysis procedures due to generalized adsorption of amino-acid-containing biomolecules including proteins.

It is generally known in the art that hydrophobic materials adsorb protein from aqueous solutions or suspensions. It is also generally known in the art that treatments to increase the hydrophilicity of materials often decrease protein adsorption. As a result, various treatments and approaches have been applied to making porous materials that have hydrophilic surfaces. Included in such approaches are methods employing application of hydrophilic polymer coatings, graft polymerizing hydrophilic monomers onto hydrophobic surfaces, treating hydrophobic surfaces with peroxides to alter surface chemistries, modifying with gas plasmas to alter surface chemistries or deposit hydrophilic plasma polymers, and blending or alloying hydrophilic polymers with hydrophobic polymers in the original preparation of the porous materials. Improvements in resistance to fouling by proteinaceous substances have been achieved in some measure by each of these approaches.

Nevertheless, the problem of protein fouling of porous materials is far from being satisfactorily solved. One of the difficulties inherent in the various approaches is the inconstancy of hydrophilicity as a surface parameter. Yasuda (*Plasma Polymerization*, Academic Press, Orlando, FL, 1985, pp. 345-354) has explained that, in polymeric materials, so-called hydrophilic surfaces contain both hydrophilic and hydrophobic molecular components, and that rotational motion inherent in most polymeric surfaces allow movement and orientation of these components so as to provide the lowest possible energy state at an interface of the polymer surface with water, air, or proteinaceous solution. Thus, a hydrogel consisting of as high as 90% water content may still exhibit a hydrophobic surface by reason of orientation of hydrophobic moieties (as evidenced by contact angle measurements), and may show significant adsorption characteristics toward proteinaceous compounds in a solution or suspension in contact with the hydrogel. A very tightly crosslinked polymeric structure having hydrophilic groups on its outer surface showed stable hydrophilicity. This was demonstrated by means of a plasma-polymerized poly(methane) layer treated

with oxygen to develop surface hydroxyl groups. Such a coating remained hydrophilic over a 200 day period. In U.S. 5,760,100 and 5,789,461, this approach has been utilized as an adjunct post-treatment in the manufacture of soft, wearable contact lenses, wherein the ocular contact surface has been treated with a methane-air mixture to provide improved hydrophilicity.

Many other approaches have been, and continue to be, developed and used to confer hydrophilicity to a material surface, including chemical grafting, chemical oxidation or etching, polymer blending or alloying, application of all sorts of polymeric coatings, and treatment with various surfactants.

A difficulty unappreciated heretofore in this field as it relates to polymeric porous materials, such as for example microfiltration and filtration membranes, is the softness of these materials when produced in their porous state. While the source polymer may be a fine example of a rigid thermoplastic engineering resin in its virgin state, processing of the same source polymer into a porous article results in an article whose surface may be easily marred by almost any kind of rubbing or abrasive contact. This softness appears to account for a portion of the hysteresis commonly observed in porous membrane materials in pressurized filtrative and concentrative applications. Previously observed hysteresis effects in filtrations have long been thought to solely reflect probable surface fouling by organic contaminants. Loss of performance in a biomaterial processing application is herein now recognized to be not solely a problem of biofouling, but also to include contribution of the softness of the porous polymeric structure by surface compaction during the pressure effects of the filtration operations. Thus, an optimum solution to the problem of biofouling of porous materials by proteinaceous matter must also take into consideration the softness and compressibility of porous polymeric materials, particularly as it relates to discriminating layers present in porous articles intended for contact and processing of biological solutions and suspensions.

It is an object of this invention therefore to provide porous materials for processing solutions or suspensions of biomaterials, wherein the porous materials have been hardened or annealed. It is a further object of this invention to utilize such porous materials in the concentration or separation of proteinaceous substances, wherein such porous materials are less adsorptive to biofoulants, particularly proteinaceous matter. It is a further object of this invention to provide improved

processes such as filtration, clarification, fractionation, pervaporation, reverse osmosis, dialysis, affinity separation, blood oxygenation, or similarly related procedures, wherein such processes are improved by means of utilization of porous materials having reduced fouling tendencies, especially to proteinaceous substances when exposed to fluids containing proteinaceous matter. These and other objects of the invention will become evident to one skilled in the art by means of the description and claims to follow.

Disclosure of Invention

It has now been found that processes for separation, fractionation, concentration, and retrieval of proteinaceous compounds, or separation of nonproteinaceous compounds admixed with proteinaceous compounds, are greatly improved by application of porous membranes to such processes, wherein the porous membranes have been treated by a plasma annealing process prior to usage in these intended applications. Thus, use of these membranes, which have a highly beneficial combination of surface hardening and hydrophilicity by means of a treatment with a gas plasma process hereinafter referred to as "plasma annealing," results in less adsorption of proteinaceous matter to exposed separation surfaces, greater throughput of permeate in filtrative and concentrative applications, and greater recovery of costly proteinaceous biologicals such as bioengineered proteins, enzymes, hormones and clotting factors.

In one preferred embodiment of the invention, porous membranes are plasma-annealed by treatment with a low temperature gas plasma generated by glow discharge through a gas containing a hydrocarbon such as an acetylene or a saturated alkane, then applied to separation or concentration of proteinaceous substances. Greatly improved throughput of permeate is achieved due to decreased membrane softness and flow hysteresis, along with a reduction in the surface adsorption characteristics of the membranes toward the proteinaceous substances.

In another preferred embodiment of the invention, porous membranes are plasma-annealed by treatment with a low temperature gas plasma generated by glow discharge through a gaseous blend containing a saturated alkane blended with air and/or a hydrophilic unsaturated monomer, then applied to separation or concentration of proteinaceous substances. Greatly improved throughput of permeate is achieved due to decreased membrane softness and flow hysteresis, along with a greatly

improved reduction in the surface adsorption characteristics of the membranes toward the proteinaceous substances. Particularly preferred for utilization in protein concentrations and separations are plasma-annealed polysulfone membranes, wherein the plasma annealing was conducted in the presence of methane blended with air, oxygen and/or acrylic acid. Such membranes exhibit greatly improved fluxes in filtration operations due to their retention of surface porosity, as well as improved surface hardness and hydrophilicity, in the plasma annealing process.

The improved processes, which are the subject of this invention, are useful in the fractionation and concentration of various proteins in fluids, including blood, biological sera, milk, and whey, as well as concentration of soy protein isolates, enzymes, bioengineered proteinaceous hormones, and such like. Concentration or fractionation of a variety of biomaterials, in solution or suspension with proteins, may similarly be practiced within this invention. Similarly, improved processes in accord with this invention are useful in the separation of nonproteinaceous substances from protein-containing fluids and suspensions.

Brief Description of Drawings

FIG. 1 is a graph of bore flow through polysulfone hollow fibers as a function of externally applied pressure.

FIG. 2 is a graph of permeate flow through polysulfone hollow fibers as a function of internally applied filtration pressure.

Best Mode for Carrying Out the Invention

In accordance with the invention being disclosed herein, utilization of plasma-annealed membranes is made in applications involving separation and/or concentration of proteinaceous substances, and in separation or concentration of biomaterials such as hormones or saccharides or nucleic acid compounds in solutions or suspensions containing proteinaceous substances, wherein porous membranes having greatly improved fluxes and flux stability along with reduced affinity for adsorption of proteinaceous substances are prepared by exposing these membranes to a glow discharge gas plasma wherein exposed surfaces are simultaneously plasma-annealed and modified by deposition of a plasma polymerizate formed in a gas or gas mixture containing at least one alkane or acetylene, preferably with a hydrophilicity-generating co-monomer.

In a method of making these improved materials, a gas or a blend of gases is

fed into an evacuated chamber, the gas or blend of gases is excited to a plasma state by a glow discharge maintained by application of energy in the form of an audiofrequency, a microwave frequency or a radiofrequency field, and a suitable substrate is exposed to the glow discharge gas plasma, whereby exposed surfaces of the substrate are modified by deposition of a plasma polymerizate. Apparatus suitable for conducting glow discharge treatments are known to one of ordinary skill in the art. Examples of such apparatus have been previously disclosed in a number of patents including for example U.S. 3,068,510, U.S. 4,147,745, U.S. 5,472,509, and U.S. 5,843,789, all of which are herein incorporated by reference. Such apparatus includes equipment suitable for continuous treatment of large batches of porous material such as rolls of sheeting, spools of fiber, belts of mounted articles, or alternate arrays of articles to be treated.

Porous materials to be treated in accordance with this invention are generally to be chosen and fashioned from organopolymeric plastics. Porosity may vary from as little as 10% to as high as 90%, defined in terms of the density of the porous materials relative to the density of nonporous resins from which the materials are fashioned. Preferably, porosity of the porous materials to be treated in accordance with this invention will vary in the range of 30% to 85%. The pores which account for the porosity of the porous materials may be open and interconnected or may be closed. For filtrative applications, such pores are preferably open and interconnected. Average pore size range for these pores, particularly those located in the surface to be plasma-annealed, will range from about 10 angstroms to about 2 microns, preferably in the range of from about 20 angstroms to about 0.7 microns. For dialytic and oxygenative applications, such pores need not necessarily be open and interconnected. The porous materials may be in the form of sheets, film, fibers, tubes, hollow fibers, porous coatings, or otherwise shaped articles.

Commonly, the matrix polymers of porous materials useful in this invention are chosen from the field of engineering resins, including aliphatic polyamides (nylons), aromatic polyamides ("aramids"), polysulfones, polycarbonates, polypropylenes, polyimides, polyetherimides, polyphenyleneoxides, polyesters, polyacrylonitriles, copolymers and terpolymers based on one or more of these polymers, and polymeric blends containing one or more of these polymers. These source polymers are routinely available in the form of pelletized engineering resins,

and many of these polymers are particularly noted for their rigidity and strength. However, processing of these polymers into porous materials, such as ultrafiltration and microfiltration membranes, commonly results in porous articles whose surfaces are soft and easily marred by almost any kind of rubbing or abrasive contact. This softness appears to account for at least a portion of hysteresis effects commonly observed in porous membrane materials in pressurized filtrative and concentrative applications and heretofore generally attributed solely to biofouling. These porous materials are often highly compressible, as well, depending on degree of porosity. In addition to this softness, these materials may at times exhibit a tendency to absorb organic components from biological solutions directly into their polymeric matrices, further exacerbating the problem of softness and pressure hysteresis.

For purposes of use in the separation and/or concentration processes of this invention, these porous materials are plasma-annealed in a gas plasma treatment apparatus generally by placing the materials in such an apparatus, evacuating the apparatus to a suitable state of vacuum, admitting a preferred mixture of gases, and initiating a glow discharge plasma through the gas mixture in a region defined be glow discharge electrodes. The glow discharge may be either generated in instant contact with the porous materials or alternatively in conjunction with subsequent passage of the porous materials through the glow discharge region.

The gas mixture is to contain an acetylene or a saturated alkane, preferably a saturated alkane, optionally with a second gas consisting of either a hydrophilic unsaturated monomer or a source of oxygen (such as air). Other gases may be present, including generally inert gases such as argon or nitrogen. By inertness herein is meant that such gases have very low tendency, or essentially no tendency, to be chemically incorporated into either the chemical structure of the porous material surfaces or into the chemical structure of any plasma polymerizate deposited onto the porous material surfaces. When it is desired to have oxygen present in the glow discharge gases, pure oxygen may be added into the vacuum apparatus, or admitted as air, or may optionally be provided in the form of an oxygen precursor instead of oxygen, insofar as such precursor (hydrogen peroxide, water, ozone, etc.) generates within the glow discharge region a significant population of the same species as would be generated by a glow discharge through a mixture containing gaseous oxygen. Air is a particularly preferred source of oxygen for the plasma annealing process, due to

its easy availability and essentially zero cost. Air is generally defined herein to include about 78% nitrogen gas and about 21% oxygen gas.

The alkane is preferably a low molecular weight, saturated hydrocarbon chosen from the group represented by methane, ethane, and propane, all of which are permanent gases at normal ambient atmospheric conditions. Particularly preferred is methane as the alkane. Methane is rather difficultly polymerized by gas plasma techniques, and a methane plasma reluctantly deposits a polymerizate at a notably slow deposition rate. Two advantages are derived thereby. First, greater exposure time may be used for plasma annealing of a porous material in a methane-based plasma, since deposition of the plasma polymerizate deposit is slow and controlled. Second, surface pores are not likely to be filled or blocked by a thick coating of plasma polymerizate, in that the deposition rate is so slow. In fact, with a methane-based gas plasma, surface pores are often slightly increased in size rather than decreased. A plasma polymerizate of methane will contain residual reactive sites, typically radical or radical-ion sites. These sites will react with air and/or contaminants subsequent to the plasma annealing process. Such sites are preferably eliminated in the current invention by simultaneous exposure to oxygen within the glow discharge zone. Oxygen reacts with these radical sites immediately upon their formation, resulting in two immediate benefits: greatly reduced residual activity, and controlled improvement of hydrophilicity.

The hydrocarbon gas may be used within the glow discharge apparatus at system pressures in the range from about 2.6 to about 130 Pa (20 - 1000 mtorr), preferably in the range from about 4 to about 26 Pa (30 - 200 mtorr). Oxygen gas may be used within the same apparatus at pressures in generally the same range as the hydrocarbon gas, generally when the hydrocarbon gas is a saturated alkane such as methane. The hydrophilic unsaturated monomer, preferably acrylic acid, may also be used within the same apparatus at pressures in generally the same range as the hydrocarbon gas. Blends of the hydrocarbon with the unsaturated organic monomer may also be used under similar conditions, a preferred example of such a blend being a mixture of methane and acrylic acid, The overall system pressure in the apparatus during the plasma annealing process may vary in the range of from about 6.5 to about 260 Pa (50 - 2000 mtorr). To maintain desired pressure levels, especially since monomer is being consumed in the plasma polymerization operation, continuous

inflow of monomer vapor to the plasma zone is normally practiced. When nonpolymerizable gases are blended with the monomer vapor, continuous removal of excess gases is accomplished by a simultaneous pumping through the vacuum port to a vacuum source. Since some nonpolymerizable gases are often evolved from glow discharge gas plasmas, it is advantageous to control gas plasma pressure at least in part through simultaneous vacuum pumping during plasma polymerizate deposition on a substrate during the plasma annealing process of this invention.

The deposition rate for a plasma polymerizate onto the porous materials from these gas blends may vary from a low of generally about one angstrom thickness per minute of exposure time to a high of generally about 100 angstroms thickness per minute of exposure time. High deposition rates are not necessarily advantageous to the plasma-annealing process, in that undesirably thick coatings may be deposited during the time necessary to achieve the annealing effect. Deposition rates can be altered by control of monomer pressure and by intensity of the glow discharge. The latter may be readily accomplished by one skilled in the art through design of the apparatus reaction chamber, placement of the electrodes, and power applied through the electrodes. In plasma annealing, it is preferred to operate at low discharge power, in the range of from about 75 watts to about 150 watts discharge power. A nominally 100 watt discharge power has been used with great success in plasma annealing of porous substrates. The time of exposure needed to achieve plasma annealing of the soft polymer surface in porous materials is in the range of about one second to about two minutes, preferably from about one second to about 20 seconds.

The glow discharge through the gas or blend of gases in the vacuum chamber may be initiated by means of an audiofrequency, a microwave frequency or a radiofrequency field transmitted to or through a zone in the vacuum chamber. Particularly preferred is the use of a radiofrequency (RF) discharge, transmitted through a spatial zone in the vacuum chamber by an electrode connected to an RF signal generator. A more localized and intensified gas plasma is attained by means of an electrode pair (one of which is a "ground"), whereas a more diffuse gas plasma is a result of a single electrode. Electrodes are preferably located exteriorly to the vacuum chamber, but may be positioned within the vacuum chamber. A rather broad range of RF signal frequencies starting as low as 50 kHz may be used in causing and maintaining a glow discharge through the monomer vapor. The 50 kHz frequency

was used with good effect in the experimental examples given at the end of this disclosure. In commercial scale usage of RF plasma polymerization, an assigned radiofrequency of 13.56 MHz may be more preferable to use to avoid potential radio interference problems.

The following examples are given: first, to illustrate the plasma annealing process as it is preferably carried out, including representative gas blends, operating conditions, and deposition rates for plasma polymerizates onto the surfaces being simultaneously annealed; second, to illustrate the utility and use of resulting plasma-annealed membranes in the concentration of proteinaceous substances such as cheese whey protein, also therewith demonstrating the hardening (i.e., annealing) effects of the plasma-annealing process and the reduced adsorptive characteristics and biofouling potential of the processed materials toward proteinaceous substances.

EXAMPLE 1

Deposition rates of plasma polymerizates onto surfaces were determined for methane and acrylic acid under plasma annealing conditions of 100 watts discharge power and varying monomer flow rates and system pressures as shown in Table 1. Deposition rates in the typical plasma processing conditions of this invention were about 0.6 to about 1.4 angstroms per second in the case of methane and about 24 to about 34 angstroms per second in the case of acrylic acid. In that very little plasma polymer deposition (i.e., about 20 angstroms) is preferred in the plasma annealing process, these deposition rates provided guidance for optimum exposure time of porous materials to gas plasmas utilizing methane or alternatively acrylic acid.

EXAMPLES 2-4

Polysulfone sheet membrane was obtained from Millipore Corporation (U.S.A.) having a product name designation of Minitan-S, and characterized as having a nominal molecular weight cut-off of 10,000 daltons. Portions of this sheet membrane weremolecular weight cut-off of 10,000 daltons. Portions of this sheet membrane were treated by plasma annealing with plasma polymerizate deposition under three sets of conditions as set forth in Table 2, at a discharge power of 100 watts. Blends of methane/air and methane/acetic acid were used in the plasma annealing process. Coating thicknesses were estimated from plasma exposure time at the process conditions, and resulting products were characterized for weak ion exchange capacity and water contact angle. A change in the hydrophilicity of the

TABLE 1

Methane flow rate (SCCM)	10	20	50
System Pressure (mtorr)	Deposition Rate (Å/sec)		
4	1.4	1.2	---
6.5	1.4	1.1	0.6
13	1.4	1.0	0.6
26	---	---	0.6
Acrylic Acid flow rate (SCCM)	19	26	35
System Pressure (mtorr)	Deposition Rate (Å/sec)		
26	24.3	33.8	32.8

treated surfaces was noted by decreased water contact angles - 30 to 50 degrees - compared with untreated membrane which showed a water contact angle of 60 degrees under identical measurement conditions.

TABLE 2

Millipore Minitan-S	Example 2	Example 3	Example 4
Gas Blend	Methane/air	Methane/air	Methane/acrylic acid
Flow Rate (SCCM)	7.5 / 2.5	10 / 40	40 / 11.8
System Pressure (Pa)	9.8	52	52
Residence Time (sec)	4.1	4.1	4.1
Estimated Coating Thickness (Å)	4.1	1.1	23
Weak Ion Exchange Capacity COOH ($1/nm^2$)	46	22	137
Water Contact Angle (degree)	50	45	30

EXAMPLES 5-9

Polysulfone sheet membrane was obtained from Osmonics Corporation (U.S.A.) consisting of an industrial ultrafiltration membrane characterized as having a nominal molecular weight cut-off of 10,000 daltons. Portions of this sheet membrane were treated by plasma annealing with plasma polymerizate deposition under three sets

of conditions as set forth in Table 3, at a discharge power of 100 watts. Blends of methane/air and methane/acetic acid were used in the plasma annealing process. Coating thicknesses were estimated from plasma exposure time at the process conditions, and resulting products were characterized for weak ion exchange capacity and water contact angle. A change in the hydrophilicity of the treated surfaces was noted by decreased water contact angles – 40 to 50 degrees – compared with untreated membrane which showed a water contact angle of 60 degrees under identical measurement conditions.

TABLE 3

Osmonics UF Membrane	Example 5	Example 6	Example 7	Example 8	Example 9
Gas Blend	Methane / Air			Methane / Acrylic Acid	
Flow Rate (SCCM)	7.5 / 2.5	7.5 / 2.5	10 / 40	40 / 13.7	40 / 14.4
System Pressure (Pa)	9.8	9.8	52	52	52
Residence Time (sec)	2.6	10.5	10.5	0.9	0.4
Estimated Coating Thickness (Å)	2.6	5.3	2.8	5.3	2.8
Weak Ion Exchange Capacity COOH ($1/nm^2$)	13	---	---	140	---
Water Contact Angle (degree)	40	---	---	50	---

EXAMPLE 10

A hollow fiber composed principally of polysulfone was obtained from Fresenius AG (Germany). It had a product name designation of PSP 600, and was characterized as having a nominal molecular weight cut-off of 30,000 daltons. This fiber was treated in a gas plasma apparatus by exposing the outer surface of the hollow fiber to a glow discharge generated in methane gas. The fiber was passed from a source spool to take-upthrough the gas plasma for a total plasma annealing exposure time of 18 seconds. The plasma-treated fiber was assembled into a hollow fiber module by conventional methods, and contained 75 fibers of 15 cm length. The fiber in the module was tested for the effect of hydraulic pressure on water permeation rate. The effect of plasma annealing was shown by means of the following type of

test. A flow rate of water was established through the bores (internal channels) of the hollow fibers, and fluid pressure was applied to the fiber exteriors by means of a peristaltic pump. For comparison, untreated (non-annealed) fibers were incorporated into the same type of module and tested under essentially identical conditions. Results of this test are shown graphically in FIG. 1 Plasma-annealed fibers were found to retained generally ideal flow through the bores throughout the pressure range employed in the test, as exemplified by the essentially straight line **10**, whereas untreated fibers showed a pronounced lower flow and pronounced curvature of the line **11** as a function of applied pressure.

In an alternate type of test, flow was established through the fibers by permeation from the fiber interior through the walls of the fiber to the exterior, results of which are graphically displayed in FIG. 2. The plasma-annealed fibers were found to retain generally ideal flow throughout the pressure range employed in the test, as exemplified by the essentially straight line **12**, whereas untreated fiber showed a moderately lower flux and a significant curvature of the line **13** as a function of applied pressure.

EXAMPLES 11-13

Polysulfone hollow fibers described in Example 10 were treated with glow discharge gas plasmas at a discharge power of 50 watts under the conditions in Table 4, using methane as an annealing gas and air (oxygen) or acrylic acid as hydrophilic plasma gases. These examples were evaluated for protein adsorption characteristics by exposing them to a streptavidin assay procedure commonly used in genomic analyses. Streptavidin-AP in tris-buffered saline solution, 0.53 nmol/liter, was applied to the modified fibers and to an untreated control to determine the degree of protein adsorption. The presence and amount of streptavidin-AP adsorbed onto the fibers was measured by chemiluminescence development techniques, counting relative light units (RLU) via a luminometer. Untreated control fiber was measured for streptavidin-AP adsorption by the same technique. Chemiluminescence readings were taken as a measure of adsorbed streptavidin-AP, and readings were compared. Examples 11 and 12 showed only 36% and 23% as much streptavidin adsorption as the untreated control fiber based on luminometer chemiluminescence readings. Example 13 showed only 6% of the level of the protein adsorption observed for untreated fiber in the streptavidin challenge, showing remarkable resistance to protein

adsorption.

TABLE 4

PSP 600 Hollow Fiber Membrane					
		Example 11	Example 12	Example 13	Control
Gas Blend		Methane/ Air	Methane/ Acrylic Acid	Methane/ Air	na
Flow Rate (SCCM)		7.5 / 2.5	40 / 13.7	7.5 / 2.5	na
System Pressure (Pa)		9.8	52	9.8	na
Residence Time (sec)		4.2	4.2	8.4	na
Est. Coating Thickness (Å)		2.1	25.6	4.2	0
Weak Ion Exchange Capac. COOH ($1/nm^2$)		27	555	47	0
Protein Adsorption (Streptavidin)	RLU	14,853	9,592	2,656	41,102
	%	36	23	6	100

EXAMPLE 14

The plasma-annealed membrane made according to the procedure of Example 9 was assembled into a spirally wound membrane filtration element by a standard approach well known in the reverse osmosis and ultrafiltration membrane industry. The spiral element had an approximate filtration area of 0.475 square meters. A spiral element was also assembled from nontreated but otherwise identical membrane for comparative purposes. The plasma-annealed and nontreated membrane elements were mounted in an ultrafiltration system and contacted with pH 6.3-6.4 cheese whey in a nonconcentrative mode (permeate returned to feed reservoir containing 400 ml cheese whey) at 50 °C, 20 psig hydraulic pressure for 120 minutes, with monitoring of permeate flow rates. The elements were then sequentially cycled through contact with cheese whey, then water, then cheese whey, then water, and finally cheese whey, each time at the same conditions of 50 °C and 20 psig hydraulic pressure for 120 minutes. Permeate fluxes for these cycles are shown in Table 5 for both the treated and untreated membrane elements. The data in Table 5 demonstrate both that the plasma-annealed membrane routinely displayed higher fluxes toward cheese whey compared to the untreated membrane, and also that the plasma-annealed membrane

showed a far more stable flux curve toward water (less hysteresis) compared to the untreated membrane.

TABLE 5

Example 14	Plasma-Annealed Membrane		Untreated Membrane	
Time (min) 1st Cycle	H_2O flux l / m^2-hr	Whey flux l / m^2-hr	H_2O flux l / m^2-hr	Whey flux l / m^2-hr
1	648	88	597	71
5	648	80	540	65
10	625	73	512	54
30	626	61	455	46
60	626	57	444	43
90	623	54	427	40
120	623	55	427	43
2nd Cycle				
1	242	65	263	48
5	228	60	242	48
10	---	57	---	46
30	219	53	230	38
60	219	51	228	38
90	213	50	222	34
120	228	48	227	34
3rd Cycle				
1	299	65	208	43
5	313	57	191	40
10	299	53	185	35
30	299	48	176	34
60	299	48	185	33
90	299	46	185	31
120	299	46	185	28

EXAMPLE 15

In the same manner as described in Example 14, a plasma-annealed membrane

EXAMPLE 15

In the same manner as described in Example 14, a plasma-annealed membrane made according to the procedure of Example 7 was assembled into a spirally wound membrane filtration element and sequentially cycled through contact with water, then cheese whey, for three consecutive cycles. Permeate fluxes for these cycles are shown in Table 6 for the plasma-annealed membrane element, and the data ratios to the untreated element data (from Table 5) are listed. The data in Table 6 again demonstrate both that the plasma-annealed membrane routinely displayed higher fluxes toward cheese whey compared to the untreated membrane, and also that the plasma-annealed membrane showed a far more stable flux curve toward water (less hysteresis) compared to the untreated membrane. The data ratios further demonstrate that the improved performance of the plasma-annealed membrane vis-a-vis untreated membrane become more pronounced with exposure time to cheese whey.

EXAMPLE 16

A plasma-annealed membrane made according to the procedure of Example 6, except that system pressure was 400 mtorr and discharge power was 50 watts. This plasma-annealed membrane was assembled into a spirally wound membrane filtration element and was sequentially cycled through contact with water and cheese whey in the same manner as in Example 15. Permeate fluxes for these cycles are shown in Table 7 along with data ratios as calculated in the manner of the previous table. The data in Table 7 again demonstrate both higher fluxes of the plasma-annealed membrane toward cheese whey compared to the untreated membrane, and also progressively improved performance of the plasma-annealed membrane vis-a-vis untreated membrane with sequential cheese whey exposure cycles.

EXAMPLE 17

The three spiral elements used in examples 14-16 were used to concentrate cheese whey. Each was operated in a concentrative mode, along with a control element containing untreated polysulfone ultrafiltration membrane. Concentrations were conducted for a three hour (180 min) period in each case. Permeate was collected and permeate flux was monitored periodically throughout the three hour runs. After each run, the elements were washed and rinsed. Then concentration runs were repeated with a fresh batch of cheese whey. Each element experienced three consecutive concentration cycles. Table 8 contains flux data for each element for each

TABLE 6

Example 15	Plasma-Annealed Membrane			
Time (min) 1st Cycle	H_2O flux l / m^2-hr	Ratio vs. Untreated	Whey flux l / m^2-hr	Ratio vs. Untreated
1	768	1.29	105	1.48
5	768	1.42	87	1.34
10	768	1.50	81	1.50
30	768	1.69	71	1.53
60	768	1.73	68	1.58
90	768	1.80	68	1.63
120	768	1.80	65	1.51
2nd Cycle				
1	356	1.35	91	1.90
5	341	1.41	80	1.67
10	---	---	77	1.67
30	313	1.36	70	1.84
60	307	1.35	65	1.71
90	313	1.41	64	1.88
120	307	1.35	63	1.85
3rd Cycle				
1	427	2.05	97	2.26
5	427	2.24	80	2.00
10	412	2.23	78	2.23
30	412	2.38	71	2.09
60	412	2.23	65	1.97
90	412	2.23	63	2.03
120	407	2.20	63	2.25

TABLE 7

Example 16	Plasma-Annealed Membrane			
Time (min) 1st Cycle	H_2O flux l / m^2-hr	Ratio vs. Untreated	Whey flux l / m^2-hr	Ratio vs. Untreated
1	782	1.31	105	1.44
5	762	1.41	87	1.39
10	739	1.44	81	1.43
30	708	1.56	71	1.54
60	677	1.52	68	1.58
90	626	1.47	65	1.58
120	626	1.47	65	1.49
2nd Cycle				
1	390	1.48	92	1.92
5	378	1.56	82	1.71
10	---	---	77	1.67
30	347	1.51	70	1.84
60	341	1.50	67	1.76
90	341	1.54	63	1.85
120	341	1.50	63	1.85
3rd Cycle				
1	370	1.78	91	2.12
5	341	1.79	80	2.00
10	333	1.80	74	2.11
30	313	1.78	65	1.91
60	327	1.77	63	1.91
90	327	1.77	61	1.97
120	327	1.77	60	2.14

TABLE 8

Time (min)	Whey flux (l / m^2-hr)				Ave. Ratio vs. Control
1st Cycle	Control	Example 14	Example 15	Example 16	
1	54	87	108	100	1.82
5	46	---	91	84	---
10	43	67	85	77	1.78
30	37	57	74	68	1.79
60	34	54	65	61	1.76
90	31	51	62	57	1.83
120	31	46	54	53	1.65
150	28	43	51	48	1.69
180	28	43	46	41	1.55
2nd Cycle					
1	40	77	100	94	2.26
5	34	65	80	80	2.21
10	31	62	74	74	2.26
30	28	54	68	68	2.26
60	26	53	63	60	2.26
90	26	48	57	57	2.08
120	24	44	54	54	2.11
150	---	41	---	51	---
180	24	---	46	48	---
3rd Cycle					
1	42	71	94	85	1.98
5	36	63	80	75	2.02
10	33	57	74	65	1.98
30	31	54	68	62	1.98
60	28	51	60	57	2.00
90	28	46	57	55	1.88
120	26	46	54	50	1.93
150	26	44	51	49	1.85
180	23	41	48	44	1.99

cycle, along with a calculated average ratio of flux for the three plasma-annealed membrane elements versus the control element. Plasma-annealed membrane elements showed from 55% to 126% more flux than the untreated membrane element during these tests. These favorable ratios were achieved even in light of the fact that the plasma-annealed membrane elements were dealing with a higher level of protein content in the latter portions of the concentration cycles than the untreated control due to their higher permeate fluxes. This is revealed by means of the data in Table 9, which show the concentration factors achieved by the plasma-annealed membrane elements. Thus, under identical processing conditions, the plasma-annealed membrane elements achieved at least a 4-fold concentration factor to as high as a 14-fold concentration factor in these cycles, compared to only a 1.8-fold concentration factor by the untreated membrane element.

TABLE 9

Parameter	Control	Example 14	Example 15	Example 16
Flux range: 3-hr whey conc. cycles (3)	54 - 23 l/m^2-hr	87 - 41 l/m^2-hr	108 - 46 l/m^2-hr	100 - 41 l/m^2-hr
Permeate volume (ml) (400 ml start volume)	132 - 174	240 - 292	325 - 352	310 - 335
Retentate volume (ml)	219 - 224	91 - 99	26 - 40	29 - 72
Whey conc. factor	1.8	4 - 4.4	10 - 15	5.6 - 14

The examples provided above illustrate how the invention utilizes plasma-annealed membranes to effect concentrations and separations, with particular examples given for treatment of cheese whey. These examples are not to be taken as limiting the nature or scope of the invention. Rather, the nature and scope of the invention is to be determined by the claims which are hereinafter joined to this description of the invention.

Claims

1. A method of concentrating a protein in solution or suspension comprising the steps of contacting a protein-containing fluid with a porous membrane, withdrawing a permeate portion of the fluid through the porous membrane, said permeate portion having a reduced content of a protein, and recovering a concentrate portion of the fluid, said concentrate portion containing an increased content of the protein, wherein the porous membrane has been previously plasma-annealed by exposing at least one surface of the porous membrane to a glow discharge gas plasma formed in a gas comprising a saturated alkane or an acetylene, wherein the exposed surface is hardened by interaction with the gas plasma concomitant with deposition of a plasma polymerizate.

2. The method according to claim 1 wherein the porous membrane has been plasma-annealed by exposure to a gas plasma formed in a gas mixture comprising a saturated alkane and at least one member chosen from the group oxygen, air, and a hydrophilic unsaturated organic monomer, said exposed surface being both hardened and rendered hydrophilic during plasma-annealing.

3. The method according to claim 2 wherein the exposed surface of the porous membrane contains pores therein having an initial average pore size, said pores having equal or greater average pore size after the plasma annealing.

4. The method according to claim 2 wherein the alkane is a member chosen from the group consisting of methane, ethane, and propane.

5. The method according to claim 2 wherein the hydrophilic unsaturated organic monomer comprises acrylic acid.

6. The method according to claim 2 wherein the protein-containing fluid comprises a milk, milk fraction, or cheese whey.

7. A method of concentrating a biomaterial in a solution or suspension containing a protein comprising the steps of contacting a fluid containing a biomaterial including a protein with a porous membrane, withdrawing a permeate portion of the fluid through the porous membrane, said permeate portion having a reduced content of the biomaterial, and recovering a concentrate portion of the fluid, said concentrate portion containing an increased content of the biomaterial, wherein the porous membrane has been previously plasma-annealed by exposing at least one surface of the porous

membrane to a glow discharge gas plasma formed in a gas comprising a saturated alkane or an acetylene, wherein the exposed surface is hardened by interaction with the gas plasma concomitant with deposition of a plasma polymerizate.

8. The method according to claim 7 wherein the porous membrane has been plasma-annealed by exposure to a gas plasma formed in a gas mixture comprising a saturated alkane and at least one member chosen from the group oxygen, air, and a hydrophilic unsaturated organic monomer, said exposed surface being both hardened and rendered hydrophilic treatment during plasma-annealing.

9. The method according to claim 8 wherein the exposed surface of the porous membrane contains pores therein having an initial average pore size, said pores having equal or greater average pore size after the plasma annealing.

10. The method according to claim 8 wherein the alkane is a member chosen from the group consisting of methane, ethane, and propane.

11. The method according to claim 8 wherein the hydrophilic unsaturated organic monomer comprises acrylic acid.

12. The method according to claim 8 wherein the biomaterial comprises a bioengineered protein, a growth hormone, a blood clotting factor, an enzyme, a milk protein, or a genomic polynucleic acid.

13. A method of separation of a nonproteinaceous compound from a protein comprising the steps of contacting a fluid comprising a nonproteinaceous compound and a protein with a porous membrane, withdrawing a permeate portion of the fluid and the nonproteinaceous compound through the porous membrane, said permeate portion having a reduced content of the protein, and recovering a concentrate portion of the fluid, said concentrate portion containing an increased content of the protein, wherein the porous membrane has been previously plasma-annealed by exposing at least one surface of the porous membrane to a glow discharge gas plasma formed in a gas comprising a saturated alkane or an acetylene, wherein the exposed surface is hardened by interaction with the gas plasma concomitant with deposition of a plasma polymerizate.

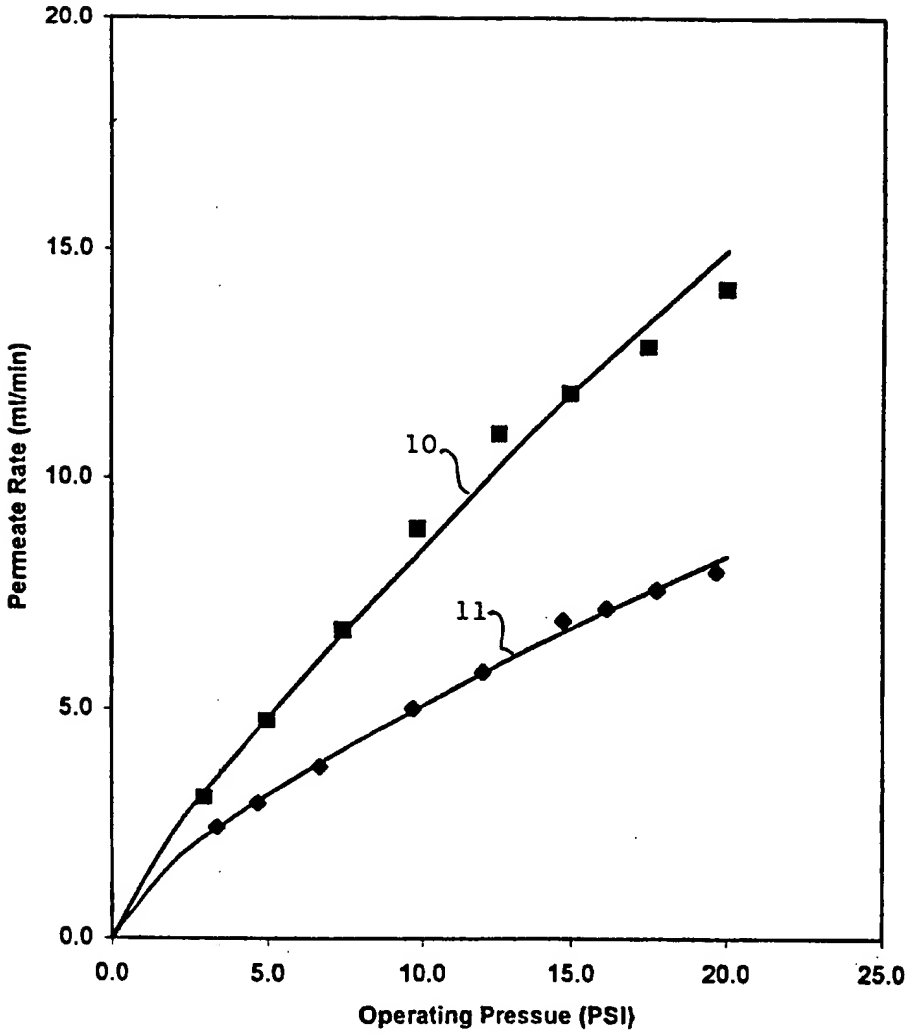
14. The method according to claim 13 wherein the porous membrane has been plasma-annealed by exposure to a gas plasma formed in a gas mixture comprising a saturated alkane and at least one member chosen from the group oxygen, air, and a hydrophilic unsaturated organic monomer, said exposed surface being both hardened

and rendered hydrophilic during the plasma-annealing process.

15. The method according to claim 14 wherein the exposed surface of the porous membrane contains pores therein having an initial average pore size, said pores having equal or greater average pore size after plasma annealing.

16. The method according to claim 14 wherein the alkane is a member chosen from the group consisting of methane, ethane, and propane.

17. The method according to claim 14 wherein the hydrophilic unsaturated organic monomer comprises acrylic acid.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15.0
10.0
5.0
0.0
Permeate Rate (ml/min)
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11
0.0
5.0
10.0
15.0
20.0
25.0
Operating Pressue (PSI)

FIG. 1

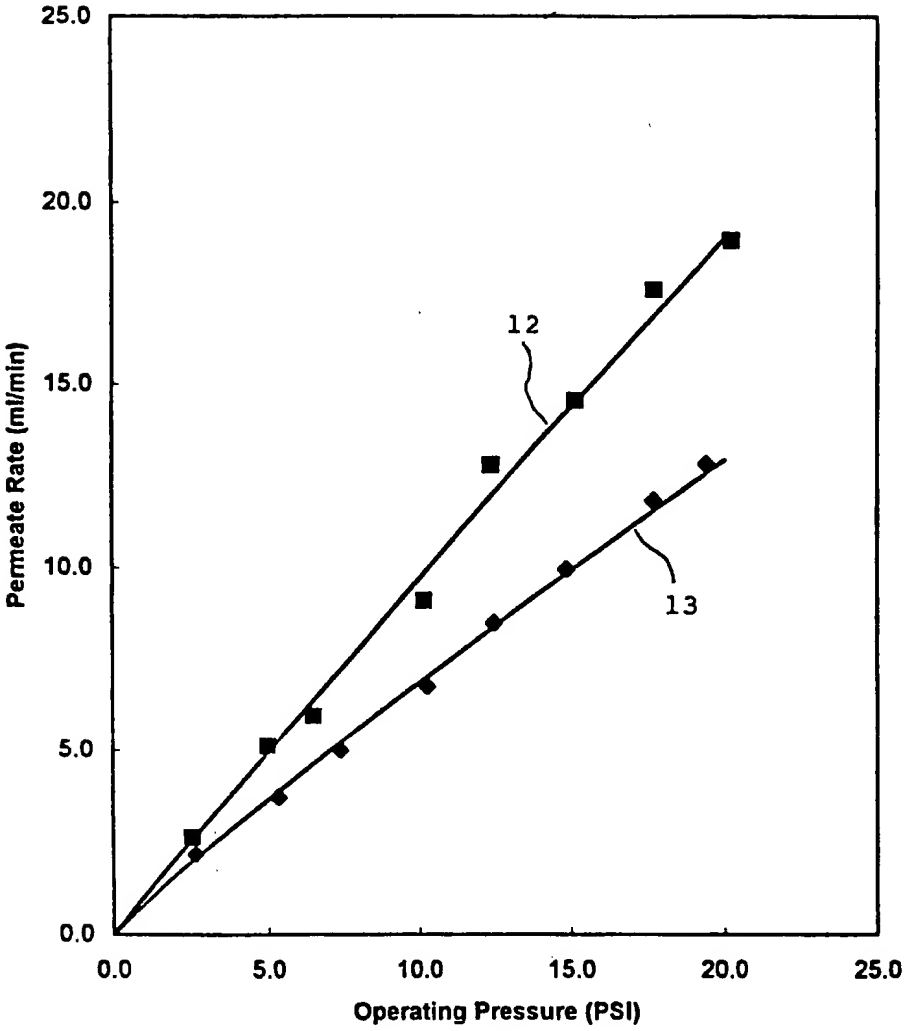
25.0
20.0
15.0
10.0
5.0
0.0
Permeate Rate (ml/min)
12
13
0.0
5.0
10.0
15.0
20.0
25.0
Operating Pressure (PSI)

FIG. 2

INTERNATIONAL SEARCH REPORT

Interna .al Application No
PCT/US 00/13550

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01D67/00 B01D69/12 B01D61/14 C07K1/34 A23C9/142
C12M1/12 C12M3/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01D C07K A23C C12M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 36877 A (NEOMECS INCORPORATED) 21 November 1996 (1996-11-21) claims 27,31,36,42; examples & US 5 843 789 A 1 December 1998 (1998-12-01) cited in the application ---	1,5,7,13
A	JOURNAL OF APPLIED POLYMER SCIENCE, vol. 47, no. 12, 20 March 1993 (1993-03-20), pages 2125-2133, XP000445562 new york,us abstract --- -/--	1,5

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 August 2000	12/09/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cordero Alvarez, M

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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